

CLAIMS

1. A mutated prokaryotic cell, which has a reduced expression-level of YusZ (SEQ ID NO's: 2 or 25), YusX (SEQ ID NO: 4), or homologues thereof, and which secretes higher amounts of at least one heterologous polypeptide of interest, when compared with an otherwise isogenic but non-mutated cell.
2. The cell according to claim 1, which is a Gram-positive cell.
3. The cell according to claim 2, which is a *Bacillus* cell.
4. The cell according to claim 3, which is a *B.alkalophilus*, *B.amyloliquefaciens*, *B.brevis*, *B.circulans*, *B.clausii*, *B.coagulans*, *B.lautus*, *B.lentus*, *B.licheniformis*, *B.megaterium*, *B.stearothermophilus*, *B.subtilis*, or *B.thuringiensis* cell.
5. The cell according to any of claims 1 – 4, wherein the YusZ or YusX homologues comprise an amino acid sequence at least 70% identical to the sequence shown in SEQ ID NO's: 2 or 25; or SEQ ID NO: 4, respectively.
6. The cell according to any of claims 1 – 5, which is mutated in *yusZ* (SEQ ID NO's: 1 or 24), *yusX* (SEQ ID NO: 3), and/or *yusY* (SEQ ID NO: 5), or homologues thereof.
7. The cell according to claim 6, wherein the *yusZ*, *yusX*, and/or *yusY* homologues encode a polypeptide having an amino acid sequence at least 70% identical to the sequence shown in SEQ ID NO's: 2 or 25, SEQ ID NO: 4, or SEQ ID NO: 6, respectively.
8. The cell according to claim 6, wherein the *yusZ*, *yusX*, and/or *yusY* homologues have a nucleotide sequence at least 70% identical to the sequence shown in SEQ ID NO's: 1 or 24, SEQ ID NO: 3, or SEQ ID NO: 5, respectively.
9. The cell according to any of claims 1 – 8, which is mutated in at least one polynucleotide, where a subsequence having a size of at least 100 bp of the at least one polynucleotide hybridizes with a polynucleotide having the sequence shown in SEQ ID NO's: 1 or 24, SEQ ID NO: 3, or SEQ ID NO: 5, or the respective complementary sequences, under medium stringency hybridization conditions.
10. The cell according to any of claims 6 – 9, in which *yusZ*, *yusX*, and/or *yusY*, or homologues thereof, is/are partially or fully deleted from the chromosome.

11. The cell according to any of claims 6 – 9, in which *yusZ*, *yusX*, and/or *yusY*, or homologues thereof, comprise at least one frameshift mutation or non-sense mutation.
12. The cell according to any of claims 1 – 11, which has at least a two-fold reduced expression-level of *YusZ* or *YusX*, or homologues thereof, when compared with the otherwise isogenic but non-mutated cell.
13. The cell according to any of claims 1 – 12, which has no measureable expression of *YusZ* or *YusX*, or homologues thereof, when compared with the otherwise isogenic but non-mutated cell.
14. The cell according to any of claims 1 - 13, wherein the at least one heterologous polypeptide comprises an enzyme.
15. The cell according to claim 14, wherein the enzyme is a lyase, a ligase, a hydrolase, an oxidoreductase, a transferase, or an isomerase.
16. The cell according to any of claims 1 – 15, which comprises one or more chromosomally integrated copies of a polynucleotide encoding the at least one heterologous polypeptide.
17. The cell according to any of claims 1 – 16, wherein the at least one heterologous polypeptide is encoded by a polynucleotide which is transcribed from at least one heterologous promoter.
18. The cell according to claim 17, wherein the at least one promoter comprises an artificial promoter.
19. The cell according to claim 18, wherein the artificial promoter comprises one or more mRNA-stabilizing sequence, preferably derived from the *cryIIla* promoter.
20. A method for constructing a mutated prokaryotic cell, said method comprising the steps of:
 - a) mutating a prokaryotic cell; and
 - b) selecting a mutated cell which has a reduced expression-level of *YusZ* (SEQ ID NO's: 2 or 25) or *YusX* (SEQ ID NO: 4), or homologues thereof, and which secretes higher amounts of at least one heterologous polypeptide of interest, when compared with an otherwise isogenic but non-mutated cell.

21. The method according to claim 20, wherein the cell is a Gram-positive cell.
22. The method according to claim 21, wherein the cell is a *Bacillus* cell.
23. The method according to claim 22, wherein the cell is a *B.alkalophilus*, *B.amyloliquefaciens*, *B.brevis*, *B.circulans*, *B.clausii*, *B.coagulans*, *B.lautus*, *B.lentus*, *B.licheniformis*, *B.megaterium*, *B.stearothermophilus*, *B.subtilis*, or *B.thuringiensis* cell.
24. The method according to any of claims 20 – 23, wherein the YusZ or YusX homologues comprise an amino acid sequence at least 70% identical to the sequence shown in SEQ ID NO's: 2 or 25, or SEQ ID NO: 4, respectively.
25. The method according to any of claims 20 – 24, wherein the cell in step (a) is mutated in *yusZ* (SEQ ID NO's: 1 or 24), *yusX* (SEQ ID NO: 3), and/or *yusY* (SEQ ID NO: 5), or homologues thereof.
26. The method according to claim 25, wherein the *yusZ*, *yusX*, and/or *yusY* homologues encode a polypeptide having an amino acid sequence at least 70% identical to the sequence shown in SEQ ID NO's: 2 or 25, SEQ ID NO: 4, or SEQ ID NO: 6, respectively.
27. The method according to claim 25, wherein the *yusZ*, *yusX*, and/or *yusY* homologues have a nucleotide sequence at least 70% identical to the sequence shown in SEQ ID NO's: 1 or 24, SEQ ID NO: 3, or SEQ ID NO: 5, respectively.
28. The method according to any of claims 20 – 27, wherein the cell in step (a) is mutated in at least one polynucleotide, where a subsequence having a size of at least 100 bp of the at least one polynucleotide hybridizes with a polynucleotide having the sequence shown in SEQ ID NO's: 1 or 24, SEQ ID NO: 3, or SEQ ID NO: 5, or the respective complementary sequences, under medium stringency hybridization conditions.
29. The method according to any of claims 25 – 28, wherein the cell in step (a) is mutated by partial or full deletion of *yusZ*, *yusX*, and/or *yusY*, or homologues thereof, from the chromosome of the cell.
30. The method according to any of claims 25 – 28, wherein the cell in step (a) is mutated by introducing at least one frameshift mutation or non-sense mutation in *yusZ*, *yusX*, and/or *yusY*, or homologues thereof.

31. The method according to any of claims 20 – 30, wherein the cell selected in step (b) has at least a two-fold reduced expression-level of YusZ or YusX, or homologues thereof, when compared with the otherwise isogenic but non-mutated cell.
32. The method according to any of claims 20 – 31, wherein the cell selected in step (b) has no measureable expression of YusZ or YusX, or homologues thereof, when compared with the otherwise isogenic but non-mutated cell.
33. The method according to any of claims 20 - 32, wherein the at least one heterologous polypeptide of interest comprises an enzyme.
34. The method according to claim 33, wherein the enzyme is a lyase, a ligase, a hydrolase, an oxidoreductase, a transferase, or an isomerase.
35. The method according to any of claims 20 – 34, wherein the cell comprises one or more chromosomally integrated copies of a polynucleotide encoding the at least one heterologous polypeptide of interest.
36. The method according to any of claims 20 – 35, wherein the at least one heterologous polypeptide of interest is encoded by a polynucleotide which is transcribed from at least one heterologous promoter.
37. The method according to claim 36, wherein the at least one promoter comprises an artificial promoter.
38. The method according to claim 37, wherein the artificial promoter comprises one or more mRNA-stabilizing sequence, preferably derived from the *cryIIla* promoter.
39. A method for producing a polypeptide of interest, said method comprising the steps of:
- a) cultivating a mutated prokaryotic cell, which has a reduced expression-level of YusZ (SEQ ID NO's: 2 or 25), YusX (SEQ ID NO: 4), or homologues thereof, and which secretes higher amounts of the polypeptide of interest, when compared with an otherwise isogenic but non-mutated cell; and
 - b) isolating the polypeptide of interest.
40. The method according to claim 39, wherein the cell is a Gram-positive cell.

41. The method according to claim 40, wherein the cell is a *Bacillus* cell.
42. The method according to claim 41, wherein the cell is a *B.alkalophilus*, *B.amyloliquefaciens*, *B.brevis*, *B.circulans*, *B.clausii*, *B.coagulans*, *B.lautus*, *B.lentus*, *B.licheniformis*, *B.megaterium*, *B.stearothermophilus*, *B.subtilis*, or *B.thuringiensis* cell.
43. The method according to any of claims 39 – 42, wherein the YusZ or YusX homologues comprise an amino acid sequence at least 70% identical to the sequence shown in SEQ ID NO's: 2 or 25, or SEQ ID NO: 4, respectively.
44. The method according to any of claims 39 – 43, wherein the cell in step (a) is mutated in *yusZ* (SEQ ID NO's: 1 or 24), *yusX* (SEQ ID NO: 3), and/or *yusY* (SEQ ID NO: 5), or homologues thereof.
45. The method according to claim 44, wherein the *yusZ*, *yusX*, and/or *yusY* homologues encode a polypeptide having an amino acid sequence at least 70% identical to the sequence shown in SEQ ID NO's: 2 or 25, SEQ ID NO: 4, or SEQ ID NO: 6, respectively.
46. The method according to claim 44, wherein the *yusZ*, *yusX*, and/or *yusY* homologues have a nucleotide sequence at least 70% identical to the sequence shown in SEQ ID NO's: 1 or 24, SEQ ID NO: 3, or SEQ ID NO: 5, respectively.
47. The method according to any of claims 39 – 46, wherein the cell in step (a) is mutated in at least one polynucleotide, wherein a subsequence having a size of at least 100 bp of the at least one polynucleotide hybridizes with a polynucleotide having the sequence shown in SEQ ID NO's: 1 or 24, SEQ ID NO: 3, or SEQ ID NO: 5, or the respective complementary sequences, under medium stringency hybridization conditions.
48. The method according to any of claims 39 – 47, wherein the cell in step (a) is mutated by partial or full deletion of *yusZ*, *yusX*, and/or *yusY*, or homologues thereof, from the chromosome of the cell.
49. The method according to any of claims 39 – 47, wherein the cell in step (a) is mutated by introducing at least one frameshift mutation or non-sense mutation in *yusZ*, *yusX*, and/or *yusY*, or homologues thereof.

50. The method according to any of claims 39 – 49, wherein the cell in step (a) has at least a two-fold reduced expression-level of YusZ or YusX, or homologues thereof, when compared with the otherwise isogenic but non-mutated cell.

51. The method according to any of claims 39 – 50, wherein the cell in step (a) has no measureable expression of YusZ or YusX, or homologues thereof, when compared with the otherwise isogenic but non-mutated cell.

52. The method according to any of claims 39 - 51, wherein the at least one polypeptide of interest comprises an enzyme.

53. The method according to claim 52, wherein the enzyme is a lyase, a ligase, a hydrolase, an oxidoreductase, a transferase, or an isomerase.

54. The method according to any of claims 39 – 53, wherein the cell comprises one or more chromosomally integrated copies of a polynucleotide encoding the at least one polypeptide of interest.

55. The method according to any of claims 39 – 54, wherein the at least one polypeptide of interest is encoded by a polynucleotide which is transcribed from at least one heterologous promoter.

56. The method according to claim 55, wherein the at least one promoter comprises an artificial promoter.

57. The method according to claim 56, wherein the artificial promoter comprises one or more mRNA-stabilizing sequence, preferably derived from the *cryIIla* promoter.